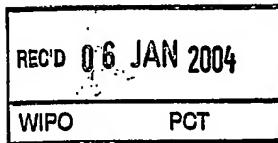




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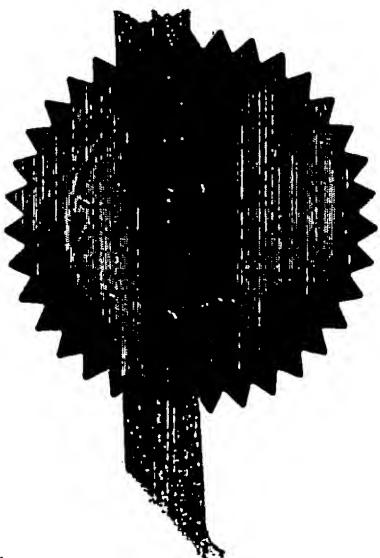
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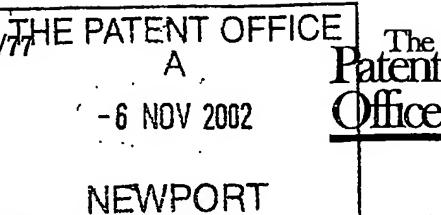


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LEEDS
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4. Title of the invention

Nucleic Acid Ligands and Uses therefor

5. Name of your agent (if you have one)

Harrison Goddard Foote

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Nucleic Acid Ligands and Uses therefor

The present invention relates to novel nucleic acid molecules or ligands with affinities for specific target molecules, and uses of such molecules, especially but not

5 particularly as diagnostic or therapeutic or screening agents, or as potential lead compounds for rationalised drug design.

Background to the Invention

10 A number of proteins undergo specific aggregation into amyloid fibrils *in vivo*, leading to a variety of pathological disorders, collectively known as amyloidoses¹. These diseases are characterised by the deposition of normally soluble proteins or peptides into insoluble fibrillar arrays. About 20 different proteins have been identified to date as the fibrillar component in different human amyloid diseases. In
15 some cases, the intact wild-type protein is involved (β 2-microglobulin, (β 2m), calcitonin, insulin and amylin), whilst in other cases (amyloid precursor protein (APP), lysozyme, transthyretin, gelsolin and others) mutated forms of the protein or protein fragments are involved. However, it is not known how normally soluble, proteins or peptides transform into the ordered array of β -strands typical of amyloid,
20 although partial denaturation of the native protein or folding of more highly denatured states is thought to be a critical first step^{2,3}. Equally importantly, the specie(s) responsible for the toxicity of amyloid is/are also unknown. There is some evidence to suggest that early oligomers or other fibril assembly intermediates, rather than the fully assembled amyloid fibrils could be the toxic agent⁴⁻⁷.

25

In recent studies, it has been shown that amyloid-like fibrils can be generated *in vitro* from proteins not known to be associated with disease⁸, suggesting that this structure could be the ultimate ground state of all polypeptide chains. In accord with this view, the sequences of proteins and peptides known to be involved in human amyloid
30 diseases also show no similarity in sequence or overall properties, for example secondary structure propensity and hydrophobicity. In all cases, the protein fibrils

formed have a common overall architecture in which β -strands orient perpendicular to the fibre long axis into an array known as a cross- β structure. Fibrils are typically long, unbranched, approximately 10 nm in diameter and are formed from simpler units known as protofilaments or filaments. Amyloid fibrils thus give rise to a 5 distinct X-ray fibre diffraction pattern, and are identified by their unique ability to bind the dyes Congo red and Thioflavin T (Thio-T), resulting in characteristic spectral changes. However, fibril formation by peptides and proteins requires specialised equipment, whilst the states of aggregation of pre-fibrillar forms of these species are difficult to detect by current methods. Therefore imaging of fibrillar 10 species in tissue samples has hitherto been difficult and largely restricted to end stage species. There is therefore a need for simple, routine screening protocols of biological tissues for all aggregated and fibrillar species.

Examples of amyloidosis disease include new variant CJD, mature onset diabetes, 15 Alzheimer's disease and dialysis-related amyloidosis (DRA). Alzheimer's disease is characterised by large plaques in the brain that contain necrotic neurons, neurofibrillary tangles containing the tau protein and fibrils composed of peptides derived from the Alzheimer's Precursor Protein (APP)⁴. With regard to the treatment 20 of Alzheimer's disease, studies with monoclonal antibodies directed against A β 1-40 have shown that neurodegeneration can be halted and/or reversed in animal models, suggesting that vaccination against A β 1-40 could be a successful therapy⁹. Unfortunately despite the promising initial animal work, recent clinical trials of this 25 therapy failed due to cross-reactivity with the APP precursor protein leading to inflammation of the brain. Accordingly there is still no effective therapy for this disease.

Dialysis-related amyloidosis (DRA) involves the human protein, β_2 m, the aggregation of which into amyloid fibrils is the cause of the disorder dialysis-related amyloidosis¹⁰. This protein forms the non-covalently bound light chain of the class I 30 MHC complex. As part of its normal catabolic cycle, β_2 m dissociates from the heavy chain, whereupon it is carried in the serum to the kidneys where it is degraded and

excreted. As a consequence of renal failure therefore, the concentration of circulating β_2m increases, and, by a mechanism currently unknown, the full-length wild-type protein aggregates to form amyloid fibrils that typically accumulate in the musculo-skeletal system. DRA is a common and serious complication of long-term 5 haemodialysis, currently affecting more than 750,000 people world-wide and serious morbidity develops in more than 90% of patients undergoing dialysis for a period of 10 or more years. Despite the identification of β_2m as the culprit protein in DRA more than 16 years ago¹¹, there are currently no therapies for the disease other than organ transplantation.

10

Accordingly, determining the structure of amyloid fibrils, together with discovery of agents able to discriminate between the differing forms of amyloid aggregates, and being able to regulate or alter their aggregation properties would offer an immediate advantage for the design of therapeutic and diagnostic agents against amyloidosis. In 15 addition, specific agents directed against either soluble Alzheimer's A β 1-40 peptide or β_2m , or their proto- or fibrillar forms, that could prevent amyloid formation and hence halt progression, and even reverse, neuro-degeneration due to plaque deposition or β_2m amyloid build up would be of immediate therapeutic value of benefit to the pharmaceutical industry and sufferers of the diseases. Furthermore, if 20 the 20 amyloidosis diseases have a common underlying molecular mechanism therapeutic reagents created against one type of amyloid could be useful against many others.

Novel synthetic DNA/RNA ligands, known as aptamers, have been defined¹² as 25 artificial nucleic acid ligands that can be generated against amino acids, drugs, proteins and other molecules. They are isolated from complex libraries of synthetic nucleic acids by an iterative process of adsorption, recovery and reamplification.

RNA aptamers are nucleic acid molecules with affinities for specific target 30 molecules. They have been likened to nucleic acid antibodies because of their ligand binding properties. They may be considered as advantageous agents over antibodies

for a variety of reasons. Specifically, they are soluble in a wide variety of solution conditions and concentrations and their binding specificities are largely undisturbed by reagents that have significant effects on antibody reagents, e.g. detergents and other mild denaturants, moreover they are relatively cheap to isolate and produce
5 compared to antibodies. They may also readily be modified to generate species with improved properties whereas antibodies cannot always be adapted easily. Extensive studies show that nucleic acids are largely non-toxic and non-immunogenic and aptamers have already found clinical application¹³, whereas antibodies being proteins are strongly immunogenic and require extensive and expensive manipulation to be
10 used in humans.

However, a serious disadvantage associated with RNA aptamers is that natural RNAs are unstable in biological fluids. It is known in the prior art how to improve stability by chemically modifying RNAs so as to block nuclease action at 5' and 3' ends, and
15 throughout the length of the molecule. However, such chemical modification can ultimately detrimentally alter the binding properties of the RNA and hence render them ineffective.

It is known from the prior art how to isolate aptamers from degenerate sequence
20 pools by repeated cycles of binding, sieving and amplification. Such methods are described in US 5,475,096, US 5,270,163 and EP0533 38 and typically are referred to as SELEX (Systematic Evolution of Ligands by EX-ponential Enrichment). The basic SELEX system has been modified for example by using Photo-SELEX where
25 aptamers contain photo-reactive groups capable of binding and/or photo cross-linking to and/or photo-activating or inactivating a target molecule. Other modifications include Chimeric-SELEX, Blended-Selex, Counter-SELEX, Solution-SELEX, Chemi-SELEX, Tissue-SELEX and Transcription-free SELEX which decsribes a method for ligating random fragments of RNA bound to a DNA template to form the oligonucleotide library. However, these methods even though producing enriched
30 ligand-binding nucleic acid products, still produce unstable products. In order to overcome the problem of stability it is known to create enantiomeric "spiegelmers"

(WO 01/92566). The process involves initially creating a chemical mirror image of the target, then selecting aptamers to this mirror image and finally creating a chemical mirror image of the SELEX selected aptamer. By selecting natural RNAs, based on D-ribose sugar units, against the non-natural enantiomer of the eventual 5 target molecule, for example a peptide made of D-amino acids, a spiegelmer directed against the natural L-amino acid target can be created. Once tight binding aptamers to this target are isolated and sequenced, the Laws of Molecular Symmetry mean that RNAs synthesised chemically based on L-ribose sugars will bind the natural target, that is to say the mirror image of the selection target. This process is conveniently 10 referred to as reflection-selection or mirror selection and the L-ribose species produced are significantly more stable in biological environments, are less susceptible to normal enzymatic cleavage and are nuclease resistant.

Statement of the Invention

15

According to a first aspect of the invention there is provided a purified and isolated non-naturally occurring nucleic acid ligand to a fibrillar protein, wherein said ligand is an RNA ligand selected from the group comprising:

- (i) the nucleic acids depicted in any one of SEQ ID NOS: 1-55 or 58 – 20 105;
- (ii) having the corresponding DNA or RNA sequences of any one of SEQ ID NOS: 1-55 or 58-105, or the corresponding fully complementary sequences thereof or their L-ribose derivatives;
- (iii) derivatives of the sequence depicted in any one of SEQ ID NOS: 1-55 25 or 58 –105 having at least about 60%, 70%, 80% or 90%, sequence identity to any one of the nucleotide sequences, and which have a binding affinity to a fibrillar protein.

Accordingly the nucleic acids of the present invention may be RNAs or their L-ribose 30 derivatives or may be the DNAs encoding the RNAs or their L-ribose derivatives.

Reference herein to fibrillar protein is intended to include all forms of the protein that is to say its monomeric, pre-fibrillar, protofibrillar and mature fibrillar forms.

Sequence identity is the similarity between two nucleic acid sequences, or two amino acid sequences, and is expressed in terms of the percentage similarity between the 5 sequences. The higher the percentage, the more similar the two sequences are. Homologues or orthologues of the protein, and the corresponding cDNA or gene sequence, will possess a relatively high degree of sequence identity when aligned using standard methods. This homology will be more significant when the orthologous proteins or genes or cDNAs are derived from species that are more 10 closely related (e.g., human and chimpanzee sequences), compared to species more distantly related (e.g. human and *C. elegans* sequences).

Reference herein to an aptamer is intended to include nucleic acid molecules with binding affinities for specific target molecules, especially but not exclusively RNA 15 nucleic acid molecules.

Preferably, the nucleic acid ligand or aptamer is substantially homologous to and has substantially the same ability to bind said fibrillar protein as a ligand selected from the group comprising the nucleic acids depicted in any one of SEQ ID NOS: 1-55 or 20 58-105.

Preferably, the nucleic acid ligand or aptamer has substantially the same structure and the same ability to bind said fibrillar protein as a ligand selected from the group comprising the nucleic acids depicted in any one of SEQ ID NOS: 1-55 or 58-105.

25 Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

30

Reference herein to binding affinity is intended to include binding affinities expressed as equilibrium dissociation constants, K_d , in the from sub-millimolar to picomolar.

5 In one embodiment of the invention, we have investigated aptamers selected against β 2m, $A\beta$ 1-40 and $A\beta$ 1-42, as both their L- and D-amino acid molecules, and their fibrillar forms. The monomeric targets being conveniently referred to as T1, the proto- and more mature fibrils as T2 and T3, respectively for the $A\beta$ targets. The equivalent β 2m targets being designated Pn, Fn and Mn. Our initial aim was to better
10 understand the molecular basis of sequence-specific recognition of RNAs by proteins and peptides. In the course of this work, we have not only isolated tight binding RNA aptamers against the targets but surprisingly and unexpectedly, the aptamers discovered have properties that make them potentially useful entities. This is in contrast to the species reported previously that bind amyloid plaques ¹⁴.

15 Accordingly, one aspect of the invention pertains to isolated nucleic acid molecules (e.g., RNAs) comprising a nucleotide sequence which has a binding affinity for a fibrillar protein or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection or
20 amplification of nucleic acid ligands for fibrillar proteins. In particularly preferred embodiments, the isolated nucleic acid molecule comprises one of the nucleotide sequences set forth in SEQ ID NO: 1-55 or 58-105 or a complement thereof of one of these nucleotide sequences. In other particularly preferred embodiments, the isolated nucleic acid molecule of the invention comprises a nucleotide sequence which
25 hybridizes to or shows at least about 50%, preferably at least about 60%, more preferably at least about 70%, 80% or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence as in SEQ ID NOS: 1-55 or 58-105, or a portion thereof.

30 Preferably, the aptamers of SEQ ID NOS: 1 to 16 have a preferential binding affinity to a D-amino acid $A\beta$ 1-40 monomeric target.

Preferably, the aptamers of SEQ ID NOS: 17 to 36 have a binding affinity to a D-amino acid A β 1-40 pre-fibrillar target.

5 Preferably, the aptamers of SEQ ID NOS: 37 to 55 have a binding affinity to a D-amino acid A β 1-40 protofibril target.

Preferably, the aptamers of SEQ ID NOS: 58 to 71 have a binding affinity to native β 2-microglobulin protein target.

10 Preferably, the aptamers of SEQ ID NOS: 72 to 90 have a binding affinity to a β 2-microglobulin immature fibril protein target.

Preferably, the aptamers of SEQ ID NOS: 91 to 105 have a binding affinity to a β 2-microglobulin mature fibrillar protein target.

15 Preferably, the aptamers of the present invention further include any one or more of the following features as herein recited such as a fluorescent label, an imaging label or a flanking region.

20 The core for the aptamer is the random RNA oligonucleotide sequence, which is flanked by a 5' and 3' constant sequence (SEQ ID NOS:56, 57, 106 and 107) that provide primer hybridisation sites for Klenow extension, cDNA synthesis, polymerase chain reaction amplification and T7 RNA polymerase transcription, all of which are involved in the SELEX protocol. It should be appreciated that the
25 selection of the constant flanking region is important to ensure optimum efficacy of SELEX. The 3' flanking region acts as the attachment site for AMV reverse transcriptase primer that converts the RNA aptamers to DNA. The 5' flanking sequence acts as the point of attachment for PCR primers which initiate amplification of the selected sequence.

According to a yet further aspect of the invention there is provided a vector comprising or encompassing at least one or more aptamer of the present invention.

This aspect of the invention pertains to vectors, preferably expression vectors, 5 containing a nucleic acid having a binding affinity for a fibrillar protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, 10 wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host 15 cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used 20 interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno- 25 associated viruses), which serve equivalent functions.

According to a yet further aspect of the invention there is provided a host cell including at least one aptamer or a vector comprising at least one aptamer of the present invention.

According to a yet further aspect of the invention there is provided an aptamer 30 binding motif comprising a peptide sequence derived from human β 2m that retains the ability of the whole protein to form amyloid fibrils. This sequence, in either the

natural L-amino acid or un-natural D-amino acid sequence, is NH₂-FYLLYYTE-COOH (SEQ ID NO: 111) or NH₂-DWSFYLLYYTEFT-COOH (SEQ ID NO:112) or NH₂-DWSFYLLYYTEFTPTEKDEY¹⁵A-COOH (SEQ ID NO:113), where the designations NH₂ and -COOH show the chemical connectivity of the peptides and the sequence in between represents the standard one-letter code for the amino acids¹⁵. Note, in each case the amino terminus of these peptides can be either free or acetylated, and likewise the carboxy terminus can be either free or in the form of an amide.

5

10 In the course of investigating the aptamers of the present invention we have identified two fibrillar forms of the protein, an intermediate, globular (immature) form and a mature fibrillar form. Accordingly this new form represents a target for identifying/developing aptamers directed thereto and may be of value in identifying further agents that can control/prevent/reverse fibril formation.

15

According to a yet further aspect of the invention there is provided aptamers directed against the binding motif as herein before described.

20 According to a yet further aspect of the invention there is provided an aptamer directed against the cross β -core structure.

There is a common secondary structure in amyloids in that whatever the fold of the soluble form they share a secondary structure core composed of a β -strand orientated perpendicular to the fibril long axis. Thus, it is envisaged that aptamers directed 25 against this target would find use as therapeutic agents in a variety of diverse amyloid diseases.

30 Currently twenty human proteins are known that cause disease via mis-folding and amyloid formation. It is estimated that amyloid formation is implicated in 1:1000 deaths in the UK and could be a major cause of tissue and organ ageing. Despite the variety of amyloid forming proteins the amyloid structure itself has a common

secondary structural element known as a cross- β -fold that appears independent of the protein/peptide sequence involved, although this does alter the detailed morphology of the mature fibrils observed. Work with antibodies has identified common epitopes (recognition features) shared by many amyloid structures. We therefore believe that

5 there is/are a common aptope(s) (aptamer recognition features) which will allow anti-amyloid aptamers to act as a generic "magic bullet". It is believed that the anti-amyloid aptamers of the present invention have the potential to cross-react with all forms of amyloid whatever its source, thus dramatically extending their utility in imaging/screening and therapeutics.

10

According to a yet further aspect of the invention, there is provided a pharmaceutical composition comprising at least one aptamer of the present invention.

15

In another embodiment of the invention the pharmaceutical composition may comprise a number of aptamers each with binding affinities for the same or different forms of a fibrillar protein.

Preferably, the pharmaceutical composition includes a suitable excipient, diluent or carrier.

20

According to a yet further aspect of the invention there is provided use of an aptamer of the present invention for the manufacture of a medicament for treating amyloid diseases.

25

It is envisaged that pharmaceuticals and medicaments utilising the aptamers of the present invention may be used to treat Alzheimer's and DRA disease conditions.

30

According to a yet further aspect of the invention there is provided a method of treating a patient suffering from Alzheimer's disease or a disease associated with amyloid formation comprising administering a therapeutically effective amount of an

aptamer or pharmaceutical composition comprising an aptamer of the present invention.

There is evidence that A β 1-40 and A β 1-42 fibril formation is naturally prevented by

5 other systems that recognise the peptide and clear it from the blood stream. These systems seem to work less well in Alzheimer's patients. If self-aggregation can be slowed and/or prevented and the growing fibrils remain soluble, it should be possible for these natural systems to keep up with peptide clearance.

10 Kidney dialysis patients suffer from induction of fibril formation in their β 2m, leading to painful deposition in joints. It is envisaged that the aptamers and pharmaceutical compositions of the present invention will be of use in treating such conditions.

15 Preferably, the aptamer or pharmaceutical composition comprising an aptamer is administered directly to an amyloid site or it may be administered by an intra-venous, intra-muscular, intra-peritoneal route and preferably may be administered on more than one occasion. In addition, aptamers could be administered to the bloodstream, the site of the raised level of soluble β 2m, in order to stabilise the soluble form of the

20 protein.

It will be appreciated that the aptamers against the A β 1-40, A β 1-42 and β 2m species may be modified with fluorescent labels by simple inclusion of, for example, fluorescein-labelled UTP in *in vitro* transcription reactions. The fluorescence properties of these molecules are sensitive to their bound state and may be the basis of simple diagnostic screening and imaging reagents. For instance, the state of disease progression may be judged by staining/screening with differently labelled aptamers directed against monomer, pre-fibril or fibrillar species and therefore preferably the aptamers and pharmaceutical compositions comprising one or more aptamers will have utility not only in treating disease conditions and ameliorating symptoms but in assaying disease prevention and progression.

According to a yet further aspect of the invention there is use of the aptamers of the present invention as a diagnostic agent for detecting the presence and/or progression of an amyloid disease.

5 Aptamers of the present invention directed against the fibrillar forms of β 2m and the reflection-selection aptamers against the A β 1-40 species or A β 1-42 species may preferably be suitably modified with at least one fluorescent label to allow diagnostic screening and/or at least one imaging reagent for these conditions. For instance, the state of disease progression may be monitored by staining/screening with differently 10 labelled aptamers directed against individual monomer, pre-fibril or fibrillar species or a mixture of species over period of time.

According to a yet further aspect of the invention there is provided a method of monitoring the presence and/or progression of an amyloid disease comprising 15 administering to a patient at least one aptamer or a pharmaceutical composition or aptamer product comprising an aptamer according to the present invention and imaging the presence of binding of said aptamer to an amyloid fibril and optionally repeating the process at a later date to assess presence or progression of a disease state.

20 According to one aspect of the invention there is provided a method for the isolation of nucleic acid ligands to a fibrillar protein target comprising:

(i) preparing a candidate mixture of nucleic acids;

(ii) contacting the candidate mixture of nucleic acids with a biotinylated 25 immobilised fibrillar protein, wherein nucleic acids having an increased affinity to the fibrillar protein relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

(iii) partitioning the increased-affinity nucleic acids from the remainder of the candidate mixture;

(iv) amplifying the increased-affinity nucleic acids to yield a mixture of 30 nucleic acids with relatively higher affinity and specificity for binding

to the fibrillar protein, whereby a nucleic acid ligand of the fibrillar protein may be identified.

5 Preferably, the candidate mixture comprises single stranded nucleic acids and more preferably the single stranded nucleic acids comprise ribonucleic acids.

10 Preferably, the target comprises at least one biotin molecule. The β 2m target protein requires biotinylation in order for it to attach to a surface, for example a streptavidin bead, so as to be immobilised however, biotinylation of native β 2m may hinder fibril formation hence we have performed this step on preformed fibrils. Our results have shown surprisingly that a combination of pH shift and biotinylation on ice does not 15 destroy fibrils and that SELEX is advantageously improved.

20 Preferably, the fibrillar target is isolated and immobilised. We have found surprisingly that, as exemplified by β 2m, increasing the solution pH > 5 does not lead to dissociation of the fibrils as expected from prevailing teachings ¹⁶, provided that the sample is maintained on ice for up to 10 minutes. Accordingly, the modified method of the present invention allows for a biotinylated target to be immobilised by pH shift.

25 Preferably, the method further includes the step of modifying the nucleic acid ligands with a fluorescent label and/or an imaging reagent. A non-limiting example of a suitable fluorescent labels is fluorescein-labelled UTP, and non-limiting example of imaging agents are uranyl acetate, and radioactive technetium and indium-labelled species, for both *in vitro* and *in vivo* applications.

30 Preferably, the method further includes the step of flanking said aptamer with at least one further nucleic acid sequence comprising the nucleic acid as set forth in SEQ ID NO:56 and optionally the aptamer is flanked by a further nucleic acid sequence as set forth in SEQ ID NO:107. It will be appreciated that other constant flanking regions

may be used and the composition of the flanking regions is not intended to limit the scope of the invention.

The method of the present invention advantageously allows for rapid selection and 5 characterisation and is accomplished *in vitro* without recourse to animal work. It will be appreciated that aptamers of the present invention will have commercial application in many areas currently making use of antibodies, for example and without limitation, as diagnostic and screening tools and as therapeutic agents in a variety of different disease conditions.

10

Preferably, the product may be further modified as hereinbefore described.

Brief Description of the Figures

15 Figure 1 represents a schematic diagram showing the growth of cross- β strand structure from soluble monomeric precursors to generate amyloid fibrils via a conformational rearrangement (top and middle lines). Also shown are models for how aptamer reagents might inhibit fibrillogenesis, either by stabilising the monomeric form (top line, right hand side) or by directly blocking fibril 20 growth(lower line).

Figure 2 shows that an anti-fibril β 2m aptamer (SEQ ID NO 74), labelled with fluorescein-UTP undergoes differential quenching and wavelength shifting of fluorescence emission in the presence of differing forms of the β 2m target.

25

Figure 3A shows surface plasmon resonance binding curves (sensograms) of the anti- β 2m fibril aptamer (SEQ ID NO 74) binding to: a) an underivatised flow cell (red, the signal falls due to differences in refractive index of the sample); b) a flow cell derivatised with native β 2m (magenta, no detectable binding under these 30 conditions); c) a flow cell derivatised with β 2m fibrils (green, strong binding with slow dissociation) or d) a flow cell derivatised with mature β 2m fibrils (blue, result

as in (c)); Figure 3B shows the equivalent experiments for a native aptamer (SEQ ID NO 61). Note, the anti-native aptamers were not counter-selected against fibrils.

Figure 4A shows a time course of A β 1-40 fibril formation followed by transmission electron microscopy (TEM) and Figure 4B a the same time course in a continuous assay utilising the increasing Thio-T fluorescence upon fibril binding.

Figure 5 A and B show nitrocellulose filter-binding curves for the binding of anti-A β 1-40 T3 aptamers (SEQ ID NO:55 and 38 respectively) to their cognate target. 5B also shows the effects of adding aptamer SEQ ID NO:38 to a mixture of A β 1-40 undergoing fibril formation (left panel). The upper TEM shows the formation of extended fibrils after 16 hours, whereas when the aptamer is added to an aliquot of the same sample after 10 mins of fibril formation further fibril formation is severely inhibited.

15

Figure 6 shows reflection-selection aptamer (SEQ ID NO:38) binding to a natural enantiomer of its initial target and indicates the potential of this bio-stable aptamer to block fibril formation.

20

Figure 7 shows mass spectrometry data for biotinylated β 2m fibrils. The data suggest that under the conditions employed 1-3 biotin residues are introduced per β 2m monomer.

25

Figure 8 shows thio-T assays of the various points of the β 2m biotin derivatisation protocol and indicates clearly that fibrils are still present after modification.

30

Figure 9 shows the nature of the fibrillar β 2m selection targets. The immature (short, curly) fibrils (left-hand side) and the mature fibrils (long, straight) on the right. The images (lower diagrams) are TEM and atomic force micrographs, respectively. The Thio-T binding potential of each fibrillar form is shown in the top panels.

Figure 10 illustrates the effects of aptamers on β 2m fibril formation *in vitro*. - β 2m in the absence of aptamers; □ - β 2m in the presence of an anti-immature fibril (SEQ: ID NO 74); ■ - β 2m in the presence of the anti-monomer 10th round aptamer pool (NR10) and as a negative control, O- β 2m in the presence of the naïve starting pool 5 for the aptamers.

Figure 11 shows the structure of human β 2m and the location of E strand target peptides.

10 Figure 12 shows the amino acid sequence of the peptides of β 2m.

Materials and Methods

- A β 1-40 and A β 1-42 Peptides

15

Fibril Formation

In order to characterise amyloid fibril formation from A β (1-40) using fluorescence assays and electron microscopy, thereby isolating and immobilising meta-stable targets for SELEX, the following method was employed. It will be appreciated that 20 the method is also applicable for amyloid fibril formation from A β (1-42) target.

0.5 mgs of unbiotinylated A β (1-40) (obtained from Biosource) was dissolved in 1 mL 99.8% trifluoroacetic acid (TFA). The TFA was then removed by passing a jet of filtered, dry nitrogen gas over the sample. The dried A β was then dissolved in 1 mL 25 hexafluoroisopropanol (HFIP, from Fluka) so as to remove any residual TFA. The HFIP was then similarly removed. The A β was then vacuum pump dried for 1 hr to remove any residual HFIP. The resulting disaggregated peptide was stored at -20°C until required.

30 10:1 'mixed' (unbiotinylated + biotinylated) A β fibrils were used from stocks of both forms of pre-prepared A β in HFIP (as described above). Appropriate volumes of each

stock were mixed to produce a ~50 μ M (10:1) mixture of unbiotinylated and biotinylated A β in HFIP. The mixture was dried under N₂ gas and vacuum pump dried to remove all the HFIP. The 'mixed' treated peptide was then dissolved in an appropriate volume of cold 1x Binding Buffer (50 mM MOPS, pH 7.4, 50 mM NaCl, 5 1 mM MgCl₂) by rapid vortexing. The peptide solution was stored on ice and then quickly transferred to a 1 mL fluorescence cuvette containing a magnetic stirrer. A volume of Thio-T solution was added to a final concentration of 5 μ M. The photobleaching shield from the fluorimeter (LS50B) was removed and a time drive scan initiated for 7200 seconds. The settings on the fluorimeter were λ_{EX} = 444 nm 10 λ_{EM} = 482 nm S_{EX} = 5 nm S_{EM} = 10 nm. The scan was followed and 40 μ L samples removed at t =100 s (corresponding to a steady fluorescence signal), 2000 s (corresponding to a slow increase in fluorescence signal), 4800 s (corresponding to an exponentially increasing in fluorescence signal); 7200 s (corresponding to a slowing of the rate of change of the fluorescence signal) and 14 400 s (corresponding 15 to the approach of saturation by the fluorescence signal). Each of these samples were negatively stained and prepared for examination by electron microscopy (Fig. 4A).

The first meta-stable precursor species to fibril formation or T2 (appeared oligomeric under the EM) were identified by a steady fluorescence signal, lasting up to 2000 20 seconds. Subsequently, T2 was prepared by stirring the A β solution for 10 minutes and then storing on ice for immediate use in SELEX experiments.

The next meta-stable precursor species to be identified were the protofibrils or T3. These remained stable for up to 30 to 40 minutes after the formation of T1 (their 25 presence was marked by a slow increase in fluorescence signal). The end of their existence is heralded by an exponential increase in fluorescence signal (marking the formation of elongating, maturing fibrils). T3 was prepared by stirring the A β solution for up to 40 minutes at 20°C and then storing on ice for immediate use in SELEX experiments. T1 or monomeric A β (1-40) was stored in HFIP and 30 subsequently diluted in 1x binding buffer when required.

With reference to Figure 4A there is shown an time course of A β 1-40 fibril formation followed by transmission electron microscopy (TEM), as will be apparent protofilaments are observed after 30-40 minutes of the procedure. Figure 4B shows a corresponding time course of amyloid fibril formation using Thio-T binding 5 fluorescence. Our results showed that both L- and D-amino acid versions of A β 1-40 exhibited the same behaviour with respect to the kinetics and morphology of fibril formation.

SELEX

10 In order to carry out at least 9 rounds of automated selection of RNA aptamers against three precursor meta-stable species of amyloid fibril formation T1(native A β); T2 (oligomeric A β); T3 (protofibrillar A β), the following protocol was adopted. Using the following reagents, a T7 MEGA shortscript transcription kit [*Ambion*], Rnase inhibitor [*invitrogen*], **Upstream primer** [5' AAT TAA CCC TCA CTA 15 AAG GGA ACT GTT GTG AGT CTC ATG TCG AA....3'] (SEQ ID NO:56), **Downstream primer** [5' TAA TAC GAC TCA CTA TAG GGA GAC AAG ACT AGA CGC TCA A...3'] (SEQ ID NO:106) and **Random 50 mer** [5' AAT TAA CCC TCA CTA AAG GGA ACT GTT GTG AGT CTC ATG TCG AA-N₅₀- TT GAG CGT CTA GTC TTG TCT 3'] (SEQ ID NO:107).

20 Each target was pre-prepared as hereinbefore described. Targets were immobilised onto Magnetic STREP-Microspheres as described by the manufacturer's instructions and stored at 4°C until required. A program Amb T1-3 was written for the automated selection of RNA aptamers against all three targets. The program was modified from 25 that of Cox and Ellington¹⁹ in the following manner:

(i) The DNA starting pool was prepared by amplifying a random 50 mer with the use of fixed upstream and downstream primer regions. The radioactively labelled pool was gel purified, quantified and subsequently 30 used to generate the first RNA pool

(ii) Ambion kit transcription reagents (transcription buffer, NTPs, T7 polymerase) (Gibco BRL) were used.

(iii) Mineral oil was used in the PCR step

(iv) Magnetic STREP-microspheres by Abgene were used instead of 5 Dynabeads

Ligation and Cloning of RT-PCR Products Generated after 9 Rounds of Automated SELEX

RT-PCR products collected at the end of Round 9 of automated SELEX were 10 amplified under the following PCR conditions: 10X PCR buffer; MgCl₂ (50 mM) to a final concentration of 1.5 mM; Taq DNA polymerase [Invitrogen] dNTP mix [Gibco BRL] to a final concentration of 0.02 mM; Upstream (SEQ ID NO:56) and Downstream primers (SEQ ID NO:106); Dimethyl Sulphoxide (DMSO); QIAEX II kit for gel purification [Qiagen]; TA TOPO Cloning system [Invitrogen].

15

Fluorescently-Labelled Aptamers

A fluorescently-labelled T3 clone RNA (aptamer against target 3) (SEQ ID NO:38) was prepared and the effect on its fluorescence signal on binding to T3 was determined. The transcription mixture was prepared as follows: 100 ng DNA 20 template 10 μ Ls; fluorescein RNA labelling mix [Roche] 2 μ Ls; 10x transcription buffer 2 μ Ls; RNase free water 18 μ Ls to 2 μ Ls; T7 RNA polymerase 2 μ Ls.

The mixture was incubated at 37 °C for 2 hrs. 2 μ Ls DNase I was then added to remove the template (incubation at 37 °C for 15 minutes). Reaction was stopped by 25 adding 2 μ Ls 0.2 M EDTA, pH8. The transcript was purified by Phenol;Chloroform extraction and desalted by passing sample through a G-25 column (Abgene). The purified fluorescently- labelled RNA was EtOH precipitated to remove all unincorporated NTPs and stored in 10 μ Ls 1x Binding buffer at -20°C until required.

30 A fluorescence emission scan (settings: λ_{Ex} = 495 nm, S_{Ex} = 3.0 nm S_{EM} = 7.0 nm) was carried out on dilute labelled RNA (1:100 1x binding buffer), a control (1 pmol

Fluorescein-12-UTP) was added and the change in fluorescence observed. Subsequently, T3 was added to labelled RNA in ratios of 1:2, 2:2, 4:2 and 6:2 and changes in fluorescence signals were observed.

Effect of T3 RNA aptamer (SEQ ID NO:38) on the Fibril Formation Assay

5 In order to determine the effect of the T3 RNA aptamer (SEQ ID NO: 38) on fibril growth assay, 600 μ Ls 50 μ M fibril growth assay as hereinbefore described was prepared. The following reagents and apparatus were used: 10 mm x 10 mm x 4 mm Styrofoam (to provide increased height of cuvette); ~440 μ g purified unlabelled transcript of T3 clone (SEQ ID NO:38); dried, purified 'Mixed' A β (1-40); 1x 10 binding buffer; magnetic stirrer; Fluorimeter LS 50B and; Formvar coated copper grids, 4%(w/v) uranyl acetate.

Fluorescence signal was observed and when the signal started to increase slowly after 30 minutes 20 μ Ls water was added and the effect observed. After a further 2-3 15 minutes the signal returned to baseline and 20 μ Ls T3.2 RNA (~440 μ gs) (SEQ ID NO: 38) was added and the effect observed. The experiment was repeated using 1/5th amount T3 RNA (SEQ ID NO: 38) (~88 20 μ gs).

• β 2-microglobulin Peptide

20

β 2m Preparation & Fibril Formation

The β 2m protein was over-expressed, extracted and purified as previously described 16. Purification was carried out using an AKTA Explorer (Amersham Pharmacia Biotech) with a HiTrap Mono Q ion exchange column.

25

Immature fibril formation was carried out using a composite buffer consisting of 25 mM TRIS, 25 mM MES, 25 mM glycine, 25 mM sodium acetate (TMGA buffer). HCl was added to adjust to pH3.6 before the addition of sodium chloride to bring the final ionic strength to 400 mM. β 2M was added to a final concentration of 1 mg ml⁻¹ 30 and the mixture incubated at 37°C for 72 hrs. Unfolding and fibrillogenesis in β 2M can be triggered by altering pH and ionic strength.

Mature fibril formation was carried out using a composite buffer consisting of 25 mM sodium acetate & 25 mM sodium phosphate. HCl was used to adjust the pH to 2.5. No additional salt was included. β_2 M was added to a final concentration of 2 mg ml⁻¹ and incubated at 37°C on a shaker 150 rpm for 7 days.

Fibrils were characterised by Thioflavin-T binding assay and electron microscopy. In both types of fibril formation reaction, a 10 μ l sample is removed and added to 990 μ l 10 μ M Thio-T buffered with 10 mM Tris HCl pH8.5. The average fluorescence 10 emission signal over a 30 second scan is then measured using a Perkin-Elmer Luminescence Spectrometer (LS50 B) set at 444 nm (excitation) and 480 nm (emission). The presence of immature fibrils is indicated by a ~10 fold increase in emission over that of Thio-T alone. Mature fibrils typically give a ~20 fold increase over that of Thio-T alone. Addition of native state β_2 M gives a marginal increase in 15 fluorescence (Fig. 8).

Fibrils of both types can be viewed using electron microscopy. Copper-coated EM grids are placed coated side down onto a 30 μ l drop of buffered fibrils for 30 seconds. Excess fibrils are removed by dotting the grid onto a 30 μ l drop of double 20 distilled water, before placing the grid onto 4% (w/v) uranyl acetate for 45 seconds. Excess stain is removed by dotting onto a fresh drop of double distilled water. The grid is then allowed to air dry before viewing using a JEOL 1200 electron microscope.

25 *Magnetic Bead Derivatisation*

Native and fibrillar β_2 M were biotin tagged using the 'EZLink' (Sulfo-NHS-LC-LC-biotin) reagent from PIERCE. The protocol supplied with the reagent had to be modified as the biotinylation reaction takes place at neutral pH (β_2 M fibrils rapidly dissociate above pH5). 'EZLink' is dissolved in DMSO to a final concentration of 1 mg ml⁻¹. 20 μ l of 1M NaOH is added to 500 μ l of preformed fibrils (~1 mg ml⁻¹) to 30 raise the pH above 6. 75 μ l of EZLink is added, mixed briefly by gentle pipetting and

incubated on ice for 15 mins. The pH is reduced and excess NHS biotin is removed by placing the entire reaction into a 'Slidalyzer' cassette (3500 Mw cut off) and dialysing against 1 L fibril formation buffer for 2 hours. The dialysis buffer is changed after 1 hour. Streptavidin coated beads, purchased from Dynal, were 5 derivatised with native β_2 M, immature fibrils or mature fibrils as described in the Dynabeads protocol.

SELEX

All rounds of *in vitro* selection were carried out on a Biomek 2000 automated 10 workstation (Beckman Coulter) using methods adapted from those described by Cox *et al.*, ¹⁸. The Biomek has an integrated PTC-200 thermocycler with heated power bonnet (MJ Research), a multiscreen filtration system & vacuum manifold (Beckman Coulter) and a Thermal Exchange Unit (Beckman Coulter) with a Thermal 48 cooling block (Acme-Automation). The Biomek and all integrated components are 15 controlled using Bioworks 3.1c (Beckman Coulter).

To produce the initial DNA pool; 20 μ l 10x PCR buffer, 1.6 μ l 10 mM dNTP's, 10 μ l 10 μ M sense primer JAN01P1 (P_1) (SEQ ID NO:108), 10 μ l 10 μ M anti-sense 20 primer JAN01P2 (P_2) (SEQ ID NO:109), 20 μ l 0.1 μ M template DNA JAN01T (SEQ ID NO:110), 6 μ l 50 mM MgCl₂, 2 μ l Taq DNA polymerase and 124.4 μ l R H₂O are thoroughly mixed and divided into 50 μ l aliquots. These are then placed into a Techne Progene thermocycler and cycled as follow; 94°C for 90 sec (Hot start), 8 cycles of: 94 °C for 45 sec (Denature); 50°C for 60 sec (Anneal) and 72°C for 90 sec (Extend)

25 As this model has a heated lid to prevent evaporation, the addition of mineral oil is not necessary. The efficiency of PCR was assessed by polyacrylamide gel electrophoresis (PAGE).

RNA Pool

Transcription reactions were carried out by adding 5 μ l 10 \times transcription buffer (to give final concentrations of 40 mM Tris-HCl pH7.9, 26 mM MgCl₂, 2.5 mM spermidine, 5 mM DTT, 0.01% Triton X-100), 16 μ l R_{H2O}, 16 μ l 25 mM NTP mix, 5 10 μ l template DNA. 40U RNasin and 100 U T7 RNA polymerase were then added to give a final volume of 50 μ l. After mixing by rapid aspiration and dispensing, the transcription mix was incubated at 37°C for 90 mins in the thermocycler.

Selection

10 20 μ l derivatised Dynabeads (approximately 6.6 \times 10⁶ beads) are added to 80 μ l binding buffer and mixed by rapid aspiration and dispensing. The binding buffer used for the selections is the same TMGA buffer used for fibril formation. In the native β_2 M selections, TMGA buffer pH7 is used. The entire transcription reaction is mixed into this. Mixing is repeated after a 5 min. room temperature incubation, to ensure the 15 beads do not settle out. Partitioning of bound from unbound species is carried out by filtration through a multiscreen 96-well plate (PVDF membrane) supplied by Millipore. The Dynabeads are then rinsed by resuspending in binding buffer and repeating the filtration. Bound RNA species are eluted by resuspending the Dynabeads in 53 μ l R_{H2O} and incubating at 95°C for 15 mins.

20

RT-PCR Amplification

The following are added to the eluted RNA; 5 μ l 10 mM primer 1, 5 μ l 10 mM primer 2, 2 μ l 10 mM dNTPs and 33 μ l RT-PCR buffer to give final concentrations of 10 mM Tris HCl pH8.4, 50 mM KCl, 5% acetamide, 0.05% Nonidet P40. This is 25 heated to 65°C for 10 mins. After reducing the temperature to 50°C, 200 U Superscript IITM reverse transcriptase and 5U Taq DNA polymerase are added. This is thermocycled as follows: 50°C for 30 mins (Reverse transcribe), 8 cycles of: 94°C for 45 sec (Denature); 50°C for 60 sec (Anneal) and 72°C for 90 sec (Extend).

10 μ l of the RT-PCR product was used as a template in the transcription reaction for the next round. After 10 successful rounds of SELEX, samples of the RT-PCR products are cloned and sequenced.

5 *Measuring Aptamer Binding Affinity by Surface Plasmon Resonance*

Individual RNA aptamers are produced by PCR amplification and gel purification of the aptamer sequence from an individual clone. This is then used as the template DNA in a 50 μ l transcription reaction (as carried out in the *in vitro* selections). Transcripts are DNase 1 treated to remove the template before phenol chloroform 10 extraction and ethanol precipitation.

All SPR experiments are carried out using a BIACore 2000. TMGA buffer pH3.6 was used as running buffer. An SA chip (Dextran matrix pre-immobilised with streptavidin) was washed by carrying out three injections of 50 μ l 'chip preparation' 15 solution (50 mM NaOH, 1 M NaCl) at 50 μ l min^{-1} to remove any loosely bound streptavidin. Each flow-cell was derivatised with a different form of $\beta_2\text{M}$ by injecting 150 μ l ~50 μM $\beta_2\text{M}$ (Native, immature or mature fibrils) across a single flow-cell at 30 μ l min^{-1} . Excess $\beta_2\text{M}$ was removed by washing the flow-cells through with a 150 μ l injection of running buffer.

20

EXAMPLE 1

Aptamers N2 and F2 (SEQ ID NOS 61 & 74) were passed across each flow-cell (30 μ l, 0.5 μM RNA at 10 μ l min^{-1}). A selection of anti-native- $\beta_2\text{m}$ aptamers were passed 25 across the blank and native $\beta_2\text{m}$ flow-cells at concentrations of 1 μM , 0.5 μM & 0.1 μM . The BIAevaluation software was used to correct the sensograms for Resonance Unit (RU) changes that are due to differences in the buffers rather than actual interactions. The software was then used to determine the Kd for each aptamer based on the corrected sensograms.

Aptamer	Kd at 1μM	Kd at 0.5μM	Kd at 0.1μM
N2 (SEQ ID NO:61)	1.09μM	1.91μM	89.1nM
N4 (SEQ ID NO:63).		104nM	317nM
N5 (SEQ ID NO:64).	785nM	300nM	845nM
N7 (SEQ ID NO:66).	2.58μM	379nM	
N8 (SEQ ID NO:67).	382nM	343nM	73.2nM
N11 (SEQ ID NO:70).	505nM	329nM	35μM
N13 (SEQ ID NO:71).	332nM	1.46μM	49.2nM

EXAMPLE 2

5 Our aptamers against the A β 1-40 and β 2m species (SEQ ID NOS: 1-55 and 58-71 and SEQ ID NOS: 72-90) have been modified with fluorescent labels by simple inclusion of fluorescein-labelled UTP in *in vitro* transcription reactions. With reference to Figure 2 differential quenching and wavelength shifting in the presence of differing forms of the β 2m target is shown with anti-fibril β 2m aptamer (F2) (SEQ 10 ID NO 74), labelled with fluorescein-UTP. The fluorescence properties of these molecules are sensitive to their bound state and may be the basis of simple diagnostic screening and imaging reagents. For instance, the state of disease progression may be judged by staining/screening with differently labelled aptamers directed against monomer, pre-fibril or fibrillar species.

15

EXAMPLE 3

Anti- β 2m aptamers have been isolated against native, monomeric protein as well as various amyloid fibrillar forms. Suitably modified versions of our aptamers against 20 the non-fibrillar forms of β 2m may be added to the blood during dialysis, stabilising that species with respect to fibril formation. Post-dialysis the aptamers will slowly hydrolyse in the body to harmless natural bi-products. Our anti-amyloid aptamers directed against β 2m, were selected at each round after counter-selection against the native monomer. Remarkably, this approach has advantageously yielded reagents that 25 bind to amyloid fibrils very tightly, dissociating very slowly, and in addition advantageously have little or no affinity for the native protein. Clearly, these reagents

can be used directly to monitor early stages of amyloidosis in patients under-going dialysis. Since the anti-native aptamers do cross-react with the amyloid forms of the protein, these results show for the first time that apatopes of the native β 2m are still present in the amyloid fibre. In addition, the fibril specific species appear to be 5 recognising amyloid-specific apatopes, suggesting that these may be common to all amyloid fibrils, massively extending their utility.

With reference to Figure 3A we have shown by surface plasmon resonance binding curves (sensorgrams) of the anti-F2 β 2m aptamer (SEQ ID NO 74) binding to: a) an 10 underivatised flow cell (red, that the signal falls due to differences in refractive index of the sample); b) a flow cell derivatised with native β 2m (magenta, that binding is detectable under these conditions); c) a flow cell derivatised with β 2m fibrils (green, that there is strong binding with slow dissociation) and d) a flow cell derivatised with mature β 2m fibrils (blue, result as in (c)).

15 With reference to Figure 3 B, we have shown by a similar method the binding of an anti-native aptamer (SEQ ID NO 61) to the same immobilised targets using SPR. The sensorgram with native β 2m as target (magenta) shows binding with rapid dissociation kinetics. This aptamer also binds strongly to the fibrillar targets 20 suggesting that the apatopes being recognised is conserved after fibril formation. In the case of the fibrils the RU response is larger because there is more protein immobilised in these cases. Also, the dissociation behaviour is clearly distinct from that seen with the native monomer, being significantly slower. This is consistent with dissociation and rebinding because of the macromolecular nature of the fibrillar 25 target.

EXAMPLE 4

We have selected aptamer sequences against the D-amino acid forms of A β 1-40. The 30 self-aggregation behaviour of these non-natural A β 1-40 peptides has been carefully assessed and three selection targets identified (Fig. 4). These are 1) T1 the

monomeric peptide; 2) T2 the pre-fibrillar state and 3) T3 the protofibrils that are the direct precursors of mature filaments. This work therefore is a significant advance over the previous report of anti-Alzheimer plaques in which natural peptides were used and their state of aggregation was not assessed. Sequence comparisons of the 5 aptamers isolated against each target suggest that there are amyloid specific aptopes in T2 and T3 that are not present in T1. The amyloid specific aptamers have some remarkable properties that make them potentially useful compounds. For example, one anti-T3 aptamer (SEQ ID NO: 38) binds its cognate target co-operatively (Fig. 5B), when labelled with fluorescence it shows specific fluorescence quenching upon 10 amyloid binding, and therefore is a potential diagnostic and/or screening agent. It also dramatically reduces fibril formation when added to an *in vitro* fibrillation assay (Fig. 6).

EXAMPLE 5

15 Experimental results with biotinylated β 2m have shown that β 2m fibrils may be biotinylated with more than one biotin molecule. With reference to Figure 7, peak A shows unbiotinylated β 2m fibrils, the 452 Da gap to peak B is indicative of a single biotin molecule and the further 452 gap to peak C that the fibril carries a second biotin molecule. Similarly, the next 452 gap to peak D illustrates that the fibril can 20 be associated with a third biotin molecule. Results have shown (Figures 8 and 9) that biotinylation of both immature and mature β 2m fibrils allows the target to be successfully attached to a bead and that biotinylation does not affect the integrity or substantially alter the properties of fibrils compared to underivatised fibrils.

EXAMPLE 6

25 Studies on β 2m fibril formation with two randomly selected aptamers F3 and NR10 (corresponding to SEQ ID NO 74 and the pool of aptamers generated after the 10th selection round, respectively) have shown profound effects on fibril formation over time (Figure 10). The anti-fibril aptamer, F3, shows distinct inhibitory properties 30 with respect to fibril formation. Strikingly, the NR10 pool appears to allow only an

initial burst of fibril formation followed by disassembly of the light-scattering species being generated, i.e. it appears capable of completely ablating fibril formation. Both aptamers were found to significantly reduce fibril formation compared to the control reaction of β 2m with the naïve pool. Interestingly, β 2m fibril formation appears to be 5 enhanced in the control sample, which may indicate that there are species in the starting RNA pool that can actively promote fibril formation. This may provide a unique insight into the natural mechanism of amyloid formation *in vivo*.

EXAMPLE 7

10

Structure of human β 2m is shown in Figure 11 and corresponding the amino acid sequence is shown in Figure 12, and is coloured from the N-terminus of the protein (red) to the C-terminus (violet). The location of elements of secondary structure was determined using DSSP. Individual β -strands are labelled A though G. The 15 disulphide bond that links Cys 25 (strand B) and Cys 80 (strand F) is also shown. The figure was made using the co-ordinates 1DUZ.PDB using the programme MOLSCRIPT and RASTER 3D. (b) The amino acid sequences of the peptides of β 2m studied here. With the exception of Ile1 and Met99, all peptide sequences were acetylated at their N-termini and amidated at their C-termini.

20

Results have shown, only two sequences, both of which encompass the region that forms strand E in native β 2m, are capable of forming amyloid-like fibrils *in vitro*. These peptides correspond to residues 59-71 (peptide E) and 59-79 (peptide E') of intact β 2m. The peptides form fibrils under the acidic conditions shown previously to 25 promote amyloid formation from the intact protein (pH < 3 at low ionic strength and pH < 5 at high ionic strength), and also associate to form fibrils at neutral pH. Fibrils formed from these two peptides enhance fibrillogenesis of the intact protein.

Accordingly, we believe that residues 59-79 are important in the self-association of 30 partially folded β 2m into amyloid fibrils and are potentially involved in the assembly mechanism of the intact protein *in vitro*. Thus, these residues represent a target

against which aptamers may be directed and these residues provide a hitherto unrecognised target site for developing amyloid disease therapeutics.

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SEQUENCES OF SELECTED REGIONS OF APTAMERS AGAINST D-AMINO ACID A β 1-40 TARGETS 1,2,3

5 Target 1 (MONOMERIC)

SEQ ID NO:1

GGA CGA AGA GCG TTA TCT TAC GCT GAC TGG ATA TTA GTT TGT TGG
AAT AC

10

SEQ ID NO:2

TCG TTA TTC TGT TCA GGG TTT GAG ACT CGT TGT ATC GAT TTA G... .

15

SEQ ID NO:3

GGC CCC TAT ACG AAC GTC ATC GTA CTC AGC TGT TGT TGT CC. . . .

20 SEQ ID NO:4

TTT ATA TCG CTT CCA CGT GTT TTA CCC ACA CCT TGT TTT AGC GGT
AA. . .

25 SEQ ID NO:5

TTA ATG ACG CTG TAC ATG GGA TCG TAT AGT GCC ACA GGT AAA TCT
GCA TG

30 SEQ ID NO:6

CTG TTC TTA TCA AGC CAA ATA AAG TAC TAT GCC CTA AGG CCT ATA
TGA TC

35 SEQ ID NO:7

CGG TGG GAA ATA TCT TTG GCT GGC TTA GTA ACT TCG TAT TTG CTC
GGC TG

SEQ ID NO:8

TTG TCC ATT GTA TAC GAT GCG CTT CAA GGA TGA TAC GGC TTT CAC
GCA ..

5

SEQ ID NO:9

TCC CTT AGC AGG CTT CAT TTC CTT GAC CAT TCT TTG CGT TGA GCC
TGT CT

10

SEQ ID NO:10

AAA CGA GTA TCT TTT TGG GAT CTT TCA GCT GCT AGT GCA ATA TCA
TCT CT

15

SEQ ID NO:12

TGG TCC TGC GAC CAG CAG TCA ATT AGG GTT TAG CAC AGT AGA
GGC CT. ...

20

SEQ ID NO:13

CGC GAC TAA CTT GCG TCA GTT TTG TAC GGG TGT TTA GCT CCC TTT
TA.

25

SEQ ID NO:14

TTC TCT CAC TTC CTT CCA GGT GAA CGG CGC ATT AAG GTC TTG TTG
AT...

30

SEQ ID NO:15

CAG AGT TAG ATG GCA GCT ATT GTC GCC TAAGTT TGC TTT ATA TCA
TCT TT

35

SEQ ID NO:16

CGG CGC GTC CCT GCA AGT CAA CTG TTT CCT GGC TCG CTT TAC GAT
CCT TT

Target 2 (PRE-FIBRILLAR)

SEQ ID NO:17

5 CGT ACA CAT CAA ACT TTC GAA TCC ACA GGA TAA TCG AGG AGA TCT
CCT GA

SEQ ID NO:18

10 TTG TCG CGA TAC TAC ATT TGT CCT GTC TGT GTG TCA TAC GGA ACT
GTG TA

SEQ ID NO:19

15 CGC CCG TCG ATT TGA AAC ACT CTT CAT CGG CGT TAA CCA CGG CGT
GGA GA

SEQ ID NO:20

20 CGT ACC GCG CTA CGT TCC CAT AAG CGT GCA ATT TGT TTG CCT TGG
GTT GG

SEQ ID NO:21

25 CTT TCA AGG TGG GGA TAC CGA GAC AAT TAG TCA TCT GTA GTGT
GAT CAG T.

SEQ ID NO:22

30 TAG AAG CGT CGA ATC ATT GGG AAT TCG TGC ATT TAG GGC AGG
CAC TTT GG

SEQ ID NO:23

35 CAA GCG AAA TGT CAC TGT GTA AGG TGT AAC AGG GAA CAA GGG
AGG ACA A.

SEQ ID NO:24

40 GGT ACT TGT TTT CGG TAC TTT GGT GCT TTG GGA CCA CGG TTT GAT
GGT GT

SEQ ID NO:25

45 TGC GCG GAG TTT CTA TTT GTG TAT GGC GTT CCT TAT CTA GGG CAG
ACT TC

SEQ ID NO:26

TTT GTA CAG TGC TTT CGC TTG TCT TCG CAG CGC GCT AAT TGC TTG
CGA GT

5

SEQ ID NO:27

GAG CTC AGT GGG GTC TTG GTA GGT TAG AAG AAG TAG CTA TGG CTT
TCG AA

10

SEQ ID NO:28

CGT GAT AGC CCA CGG TCA AGG GTC TAC GAG TCA TTT GTT CCT AAC
GCT GC

15

SEQ ID NO:29

CAA GGA GGA TAT ATG CTT GGG ATC AGT CGT GTT GAT ATT TAT
GAT GG

20

SEQ ID NO:30

AGG CTG CAG GCT TGG ATG GTG CTT CGG GGC GTC GTC TTA TTG CCT
GCT AC

25

SEQ ID NO:31

GCC TCG GTG TGT TAA GGA GGA AGA GCG CCC ATC TCA GCA GTT
ACC GGT CG

30

SEQ ID NO:32

CGC CCC GTC GAT TTG AAA CAC TCT TCA TCG GCG TTA ACC ACG GCG
TGG AG

35

SEQ ID NO:33

CGG ACT GAG CAT TAG TTC GTG TCA CAG CGA CGT CGT TGT GTA AGG
CTG AA

40

SEQ ID NO:34

TCC TGC CAA GCT CCT TTT TTT GGT AGT GAA TTT CCT TTA TCC TGG
GGC GA

SEQ ID NO:35
CTA AAG TAT GTG TTA GTA TGG CCC TGC TTA TTA ATG TCG GGA GTT
CGG ..

5

SEQ ID NO:36
TAG AAG CGT CGA ATC ATT GGG AAT TCG TGC ATT TAG GGC AGG
CAC TTT GG

Target 3 (PROTOFIBRILS)

SEQ ID NO:37

TAA AGT GCG GAC TCA GGG AAT AAG GTG AGA ACT GAC CAA GGT
5 GTG TGT TG

SEQ ID NO:38

TCG ACT TTT GAC AAG CAT CTC TTT TAC TGA GAG TTG GGA GGG AAC
10 GGA TG

SEQ ID NO:39

GGG GTG CTA AGC ATT TGG CAT CCG TTT TGT GAA CTG CTC GAT GTT
15 TAT TC

SEQ ID NO:40

TTA ACT GCA CCT TGC AGT AAC TAC AGG ATA GCA GTC CTA GAG CCA
20 GTC AT

SEQ ID NO:41

TGC ATT ACC AGG GGA TAA CCT CGC ATT TGG CGG GTT AGG AGT AAC
25 GTG CA

SEQ ID NO:42

ACT CTC GGT ATA TTG TTA GAT TGG GAG CGA CGC GTC ACC GTA
30 TGA CAT GT

SEQ ID NO:43

GGT TAG CGG GGT TAC CTA ACA TCA GCT CTA GTT AGA CCT TTT TTT
35 ACA AC

SEQ ID NO:44

CGA ATG CTT CGT AGT AAG TAA TGA ATC TTT TTG ACA CAC TAA TCG
40 TAG GA

SEQ ID NO:44

GTC CCG TCC ATA GTG CTC GCC ACC ATT TCC TTT GGG TCA GGA ATT
45 AGT TC

SEQ ID NO:45

TGG GAG AAG GAC TAG CTC GTT ATT GGT CAT GTT AGT CAT AGC CTG
ACA CC

5

SEQ ID NO:46

CGG GGC ATA GTA CAG AAT CTT AGT GGG TGT TCT TTG ATT CTG GAG
GAG CA

10

SEQ ID NO:47

GTG TGT TTT TAT CTA CTG GTC GGT GGT TTG GTT GGT AAT GCT TAA
GCG GC

15

SEQ ID NO:48

TTG GAA CCC AGT GTG TTT TAC ACG CTC ATC TTT GCA ACA GGG TGT
GTG AC

20

SEQ ID NO:49

ACG GTT AAC AAT CAT GGG CAA GCT CTT AGA ACA GCG TTC TGC GAT
TTA CGA

25

SEQ ID NO:50

GAC GCC GCC GGG GAG CTC TAA ACG GAT TAC CAT TTG TCG GTG GTC
TTA TT

30

SEQ ID NO:51

GCA TAG GAT TTC GGT TGG GAT AGA TAT TAT TCG GTT TCG ATC ATA
CGT AT

35

SEQ ID NO:52

CTC ATA GGG ATG GTC TCC CGC TGG GCT TTT TCT GTT AAC ATT TAG
AAC TC

40

SEQ ID NO:53

CAG GCC GCC GGG GAG CTC TAA ACG GAT TAC CAT TTG TCG GTG GTC
TTA TT

SEQ ID NO:54
TCA CGT GTG CTG TAC GAC TGA TGT TTT TGC TGC CAG AATG ACT
AGA AAT GT

5

SEQ ID NO:55
ATC GTA AGT GAT TGT TGT TTT ACA TGG CCT CCA GTT GGG GAG TCT
TCC AC

10

SEQUENCES OF CONSTANT FLANKING REGIONS (AT 5' AND 3'ENDS) OF INSERTS

15

5' end of top strand (primer 1) SEQ ID NO:56
5' AAT TAA CCC TCA CTA AAG GGA ACT GTT GTG AGT CTC ATG TCG
AA.....

20

5' to 3' end of top strand (complementary of primer 2) SED ID NO:57
.....TTG AGC GTC TAG TCT TGT CTC CCT ATA GTG AGT CGT ATT

SEQUENCES OF SELECTED REGIONS OF APTAMERS AGAINST β 2m.

Primer 1 SEQ ID NO:58

AGTAATACTGACTCACTATAGGGCCAAGCTTGCATGCCTGCAG

5

Primer 2 SEQ ID NO:59

GGTACCGAGCTCAGGTTCCC

10

SEQ ID NO:60

NATTAGGCGAACATAGTAGTGANAGAAAGAAAGNACAGCANGAGGGCT

15

SEQ ID NO:61

GTTGGTTACGCGTACAGTGAAACTCGGAAAGTTGAAGGGCAAATGGGAAC

20 SEQ ID NO:62

GAGCAGCACCGGGCACGGCGAACCTAAATCGGAGCCGGGTGCAGCATGTGAC

25 SEQ ID NO:63

GGCNTGACANGGCAGGATAGCCGGNCANGCGANCNTNGNCCNCANACCCGACNGTGG

25

SEQ ID NO:64

GGTACCGAAAAGTCATTGGTTACAACCAGGCAGGCCAGGAA

30

SEQ ID NO:65

GAATGGGCGAGTTGAGCGACTGAGGCAGCATGGAATCATACGTAC

35

SEQ ID NO:66

GGAGGCCGAGGGCAGAACCCAGCGACCGACGGAAAGACCATAACGGCAGC

40

SEQ ID NO:67

GCACACGGGCCACCCCTGCGAAACGACTATACGTCACTAGAGCAGGTTGGGAGTCCC

45

SEQ ID NO:68

GTGCGAGCAGGGCAACCAGAAAGAAAACGTAGGTAGAGTGTGAGTTGATGAGACGT

50

SEQ ID NO:69

GCCGCAAGAACGGGAGAAGAGCGGAGAACGTTGAACCGACCGGAGG

55

SEQ ID NO:70

GTAGAGTGTGGCTAGCGTGACCAGACATCTACGTGGCAGGGAGCATTG

55 SEQ ID NO:71

GGAGGGGCCGTAAACCGAACGAGACAACCAGATAAGGACCGCCAATGAGAGGAACGGCAA

pH3.6 induced β 2m fibrils (Immature Fibrils)

SEQ ID NO:72
5 AGTAATACGACTCACTATAGGGCCAAGCTTGCATGCCTGCAGATGAGAATACAGAACAGAGGAGAA
GGGGATGAATCAATAAGGTACACTAACCAACNCGGTACCGAGCTCAGGTTCCC

SEQ ID NO:73
10 ATGAGAATACACAGAACAGAGGAGAAGGGGATGAATCAATAAGGTACACTAACCAACNC

SEQ ID NO:74
15 AAAATAAANTGTGTACACNAAANNGCNANCCCNNGAANGAANGNCAACAAANGTNAGT

SEQ ID NO:75
20 GGGAGAAATTATGGAGTTGTAAAACCATTGGTCAGTGGCAGAGGTTCATCACCGTCATC
ACACCTGGAATTAAATTCAAGGACACCCAACGCCCTACGAATAGAGCGGGAACGACGACC

SEQ ID NO:77
25 CGGAAGAGCACAAAAAGGGGACTCGCCTTGGAAAGGAACNTGC

SEQ ID NO:78
30 TTACGTCNGGCTNAACGAATGAACGGGAAAGNAAGGAATGATTGGANCATANACAGN

SEQ ID NO:79
35 GGAGTGTGGATATGA

SEQ ID NO:80
40 ACACGGAGGCACCTACAGTACTGGGCAGGAGTTGCGCAGAGGACTAGTGGAAAAGGAGT

SEQ ID NO:81
45 GCCAGCAGGACAAANAGCAGGACGTTAAGAAGACGAAAGGGAGAACATTGGGGAGACGA
CCTGGGATCTCCAGGGGGCGGGAGCGATAGGTGTGCGTGAGTTGAAAAGGCAGGGCAA

SEQ ID NO:83
50 GAGACAAGTCGGTAGGACAGAGGAGTGAACAAAGAAGCAGAGGAGAGTCAGAGCAGC

SEQ ID NO:84
55 ATCTACCGGTTACAAATCAAGAGAACTATTTGTATGAGCCACTTATCTCTCTGAAGT

SEQ ID NO:85
60 CGCANGAAATATAACGTGGGAGGACCAGGGAGAGTACTCCAAAGAGTGAAGAAGGTT
GCGGTAGAATGCATGGCGATTGCGATTGTAGGATTCCGCATCACACCTCC

SEQ ID NO:87
AAAAGGACAGGAGC

5 SEQ ID NO:88
ACTATAAGTNAGNNGGAGGGGGANCATGAAACAATNGTTAAGAACGTANANTGGNTGA

10 SEQ ID NO:89
ACACGGAGGCACCTACAGTACTGGGGCAGGAGTTGCCAGAGGACTAGTGGAAAAGGAGT

15 SEQ ID NO:90
AACGAAATGATAACAACAAATACACACGTCAACCGAAGTTGACCCAAGACTC

Aptamers against β2m mature Fibrils

SEQ ID NO: 91
5 ATTAACGAGANTGAAGGAAANC GGAGGAGANCAGGCACAACGGGACANGAAAAGAGAGAGTGC GG
TANCCG

SEQ ID NO: 92
10 CCGAGGCCGCACACATAAGGGGGAGGGAAAGACAGCAGAATAANAGGAGGCCATAGTNC

SEQ ID NO: 93
15 GGTAAGGTGGAAGGGAAAAGGAAACGGAATGAACCCGCGTTATGGCCGAAAAGGGAC

SEQ ID NO: 94
20 GAACAGGACGCGTAGCCCTACGATCCA

SEQ ID NO: 95
25 CGAGGACGAAANAGTTAGAAAAGGAGACGGCGCTTCGAACGAACCATCCCCTAGGT

SEQ ID NO: 96
30 AAGTGANNAGGAAAAGGAAAAAAAAGGTGAACAAGGCTGCTC

SEQ ID NO: 97
35 GGGCCGACGAAGTGGGGGAGGAACGTTNGTGTGAGTGATTGGAGTNCCCTGGCGAAATCC

SEQ ID NO: 98
40 GCAAACGGGGCGTTCCGGGGAGGAGTGGGAAGAAAACGAGAGAAGAGTTGGAACG

SEQ ID NO: 99
45 GCANGGGAANGGATTGAACGAGAAGAAGGAGGAGC

SEQ ID NO: 100
50 GCACCTTCTCACTTNNGGCACAAAACCGCGCACCGGGCTAGNCTGGAGTGGATGAACGAC

SEQ ID NO: 101
55 TGAGGGGAGGTTGGCGCGAAGGCGAGGCAGCAGCAGAACAGTTTGAGTCGAGAGGGAT

SEQ ID NO: 102
60 CCTGGAGGAGGGCAGCGGNCAGAAGGGAAAAAGAAAAGAACTATTGTGGAGCGGAT

SEQ ID NO: 103
GAAAAGGAGGGATTNAAATCGCGTGAAGGTGAATTGTTGCCAGGGCGAATNCGGT

SEQ ID NO: 104
65 GGGGCTTCATNGAGAGAACGACAGCAGAAAAAGGGGGGNGTTCAAGAGGAAGAGACCGA

SEQ ID NO: 105
70 GTGTAAGCTTGCCTCGAAGATGAANAAAATGTCGAATCCTCGCTGTGCCGCCAGACGTGA

Downstream primer (SEQ ID NO:106)

[5' TAA TAC GAC TCA CTA TAG GGA GAC AAG ACT AGA CGC TCA A...3'] (SEQ ID NO:106)

5

Random 50 mer (SEQ ID NO:107).

[5' AAT TAA CCC TCA CTA AAG GGA ACT GTT GTG AGT CTC ATG TCG AA-N₅₀- TT GAG CGT CTA GTC TTG TCT 3'].

10

JAN01P1 (SEQ ID NO:108)

5'AGT ATT ACG ACT CAC TAT AGG GCC AAG CTT GCA TGC CTG C^{3'}

JAN01P2 (SEQ ID NO:109)

15 5'GGG AAC CTG AGC TCG GTA CC^{3'}

JAN01T (SEQ ID NO :110)

5'CCA AGC TTG CAT GCC TGC AG N₆₀ GGT ACC GAG CTC GAA TTC CC^{3'}

20 SEQ ID NO: 111

FYLLYYTE

SEQ ID NO:112

DWSFYLLYYTEFT

25

SEQ ID NO:113

DWSFYLLYYTEFTPTEKDEYA

Claims

1. A purified and isolated non-naturally occurring nucleic acid ligand to a fibrillar protein target, wherein said ligand is an RNA ligand selected from the group comprising:
 - (i) the nucleic acid depicted in any one of SEQ ID NOS: 1-55 or 58-105;
 - (ii) having the corresponding DNA or RNA sequences of any one of SEQ ID NOS: 1-55 or 58-105 or the corresponding fully complementary sequences thereof or their L-ribose derivatives;
 - 10 (iii) derivatives of the sequence depicted in any one of SEQ ID NOS: 1-55 or 58-105 having at least about 60%, 70%, 80% or 90% sequence identity to any one of the nucleotide sequences, and which have a binding affinity to a fibrillar protein.
- 15 2. The nucleic acid ligand according to claim 1 which is substantially homologous to and has substantially the same ability to bind said fibrillar protein target as a ligand selected from the group comprising the nucleic acids depicted in any one of SEQ ID NOS: 1-55 or 58-105.
- 20 3. The nucleic acid ligand according to either preceding claim which has substantially the same structure and the same ability to bind said fibrillar protein target as a ligand selected from the group comprising the nucleic acids depicted in any one of SEQ ID NOS: 1-55 or 58-105.
- 25 4. The nucleic acid according to any preceding claim wherein the fibrillar protein target is selected from the group comprising monomeric β 2m or $A\beta$ 1-40 or $A\beta$ 1-42, protofibrillar β 2m or $A\beta$ 1-40 or $A\beta$ 1-42, mature fibrillar β 2m or $A\beta$ 1-40 or $A\beta$ 1-42.
- 30 5. The nucleic acid according to any preceding claim wherein the fibrillar protein target comprises either L- or D-amino acid molecules.

6. The nucleic acid according to any preceding wherein the nucleic acid of any one of SEQ ID NOS: 1 to 16 have a preferential binding affinity to a D-amino acid A β 1-40 monomeric target.

5 7. The nucleic acid according to any one of claims 1 to 5 wherein the nucleic acid of any one of SEQ ID NOS: 17 to 36 have a preferential binding affinity to a D-amino acid A β 1-40 pre-fibrillar target.

10 8. The nucleic acid according to any one of claims 1 to 5 wherein the nucleic acid of any one of SEQ ID NOS: 37 to 55 have a preferential binding affinity to a D-amino acid A β 1-40 protofibril target.

15 9. The nucleic acid according to any one of claims 1 to 5 wherein the nucleic acid of any one SEQ ID NOS: 58 to 71 have a preferential binding affinity to a native β 2-microglobulin protein target.

10. The nucleic acid according to any one of claims 1 to 5 wherein the nucleic acid of any one of SEQ ID NOS: 72 to 90 have a preferential binding affinity to a β 2-microglobulin immature fibril protein target.

20 11. The nucleic acid according to any one of claims 1 to 5 wherein the nucleic acid of any one of SEQ ID NOS: 91 to 105 have a preferential binding affinity to a β 2-microglobulin mature fibrillar protein target.

25 12. The nucleic acid according to any preceding claim further including any one or more of the following features:

- (i) a fluorescent label;
- (ii) an imaging label or;
- (iii) a flanking region.

13. The nucleic acid according to claim 12 wherein the flanking region comprises any one or more nucleic acid sequences selected from the group comprising SEQ ID NOS: 56, 57, 106 and 107.

5 14. A vector comprising at least one or more nucleic acid as defined in any preceding claim.

15. A host cell including at least one or more nucleic acid as defined in any of claims 1 to 13 or the vector of claim 14.

10 16. Use of a binding motif comprising a peptide sequence derived from human β 2m that retains the ability of the whole protein to form amyloid fibrils, as a target for selecting a nucleic acid ligand.

15 17. Use of a peptide sequence comprising any one of SEQ ID NO: 111, 112 or 113 or derivatives or variants thereof that retain the ability of the whole protein to form amyloid fibrils, as a target for selecting a nucleic acid ligand.

20 18. A purified and isolated non-naturally occurring nucleic acid ligand to a fibrillar protein target, wherein the target comprises a binding motif as defined in either claim 16 or 17.

25 19. A purified and isolated non-naturally occurring nucleic acid ligand to a fibril cross β -core protein target.

20. A pharmaceutical comprising at least one nucleic acid as defined in any one of claims 1 to 13 or the vector of claim 14.

30 21. A pharmaceutical according to claim 20 comprising a number of nucleic acid ligands each with binding affinities for the same or different forms of a fibrillar protein.

22. A pharmaceutical according to either claim 20 or 21 further including a suitable excipient, diluent or carrier.
23. Use of a nucleic acid according to any one of claims 1 to 13 for the manufacture of a medicament for treating amyloid diseases.
24. Use according to claim 23 for the treatment of Alzheimer's and DRA disease conditions.
- 10 25. A method of treating a patient suffering from Alzheimer's disease or a disease associated with amyloid formation comprising administering a therapeutically effective amount of a nucleic acid ligand according to any one of claims 1 to 13, or the vector of claim 14 or a pharmaceutical according to claims 20 to 22.
- 15 26. A method according to claim 25 wherein the therapeutically effective amount of a nucleic acid ligand or vector or pharmaceutical is administered by an intravenous, intra-muscular, intra-peritoneal route and optionally is administered on more than one occasion.
- 20 27. Use of the nucleic acid according to any one of claims 1 to 13 or the vector of claim 14 as a diagnostic agent for detecting the presence and/or progression of an amyloid disease.
28. A method of monitoring the presence and/or progression of an amyloid disease comprising administering to a patient at least one nucleic acid according to any one of claims 1 to 13 or the vector of claim 14 or a pharmaceutical according to any one of claims 20 to 22 and imaging the presence of binding of said nucleic acid ligand to an amyloid fibril and optionally repeating the process at a later date to assess presence or progression of a disease state.

29. A method for the isolation of nucleic acid ligands to a fibrillar protein target comprising:

- (i) preparing a candidate mixture of nucleic acids;
- 5 (ii) contacting the candidate mixture of nucleic acids with a biotinylated immobilised fibrillar protein on ice, wherein nucleic acids having an increased affinity to the fibrillar protein relative to the candidate mixture are partitioned from the remainder of the candidate mixture;
- (iii) partitioning the increased-affinity nucleic acids from the remainder of the candidate mixture;
- 10 (iv) amplifying the increased-affinity nucleic acids to yield a mixture of nucleic acids with relatively high affinity and specificity for binding to the fibrillar protein, whereby a nucleic acid ligand of the fibrillar protein may be identified.

15 30. A method according to claim 29 wherein the candidate mixture comprises single stranded nucleic acids.

31. A method according to claim 30 wherein the single stranded nucleic acids comprise ribonucleic acids.

20 32. A method according to any one of claims 29 to 31 further including the step of modifying the nucleic acid ligand with a fluorescent label and/or an imaging reagent.

25 33. A method according to any one of claims 29 to 32 wherein the nucleic acid ligand further comprises a flanking selected from the group comprising SEQ ID NO: 56, 57, 107 or 108.

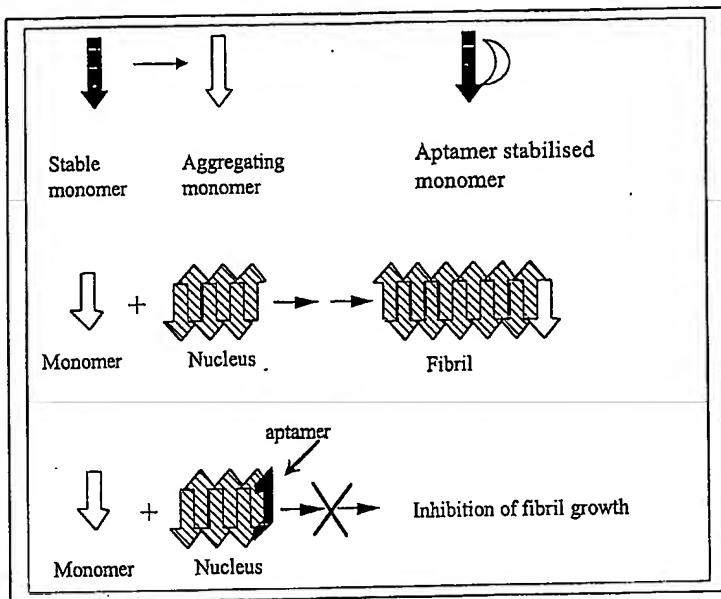
34. A nucleic acid product identified and isolated according to the method of any 30 one of claims 29 to 33.

FIGURE 1

5

10

15



1 F2 : 100 B2M

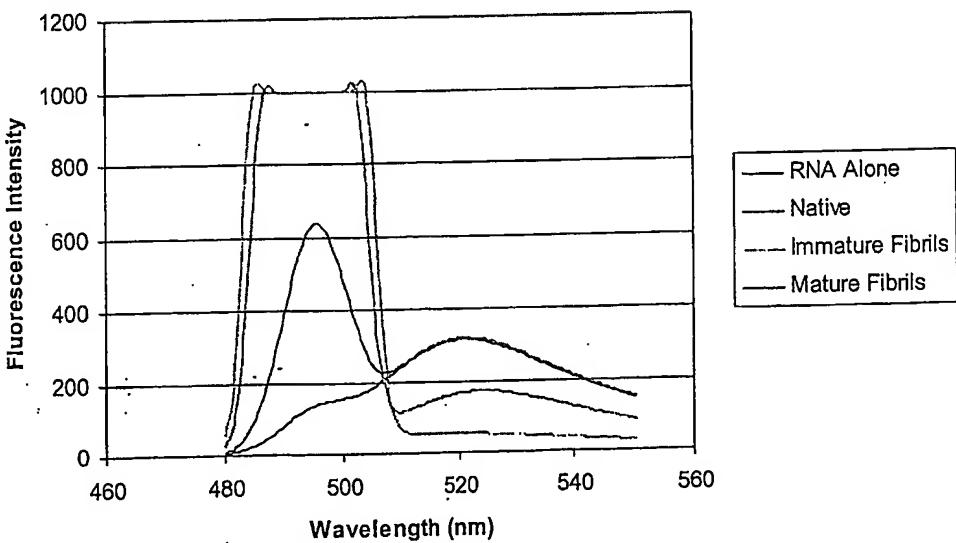
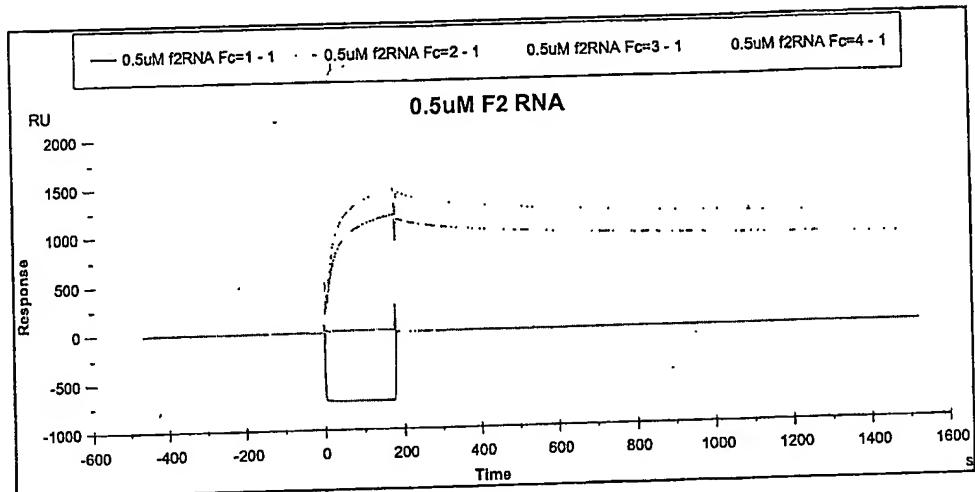


FIGURE 3A



5

FIGURE 3B

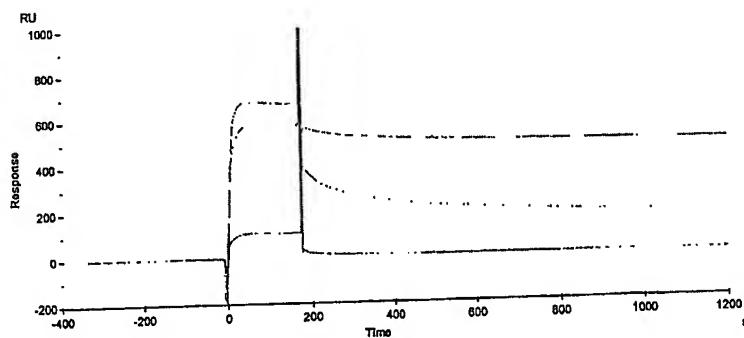
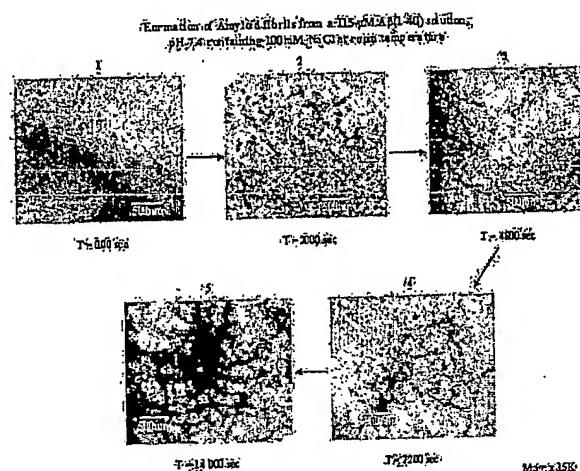


FIGURE 4A



5

FIGURE 4B

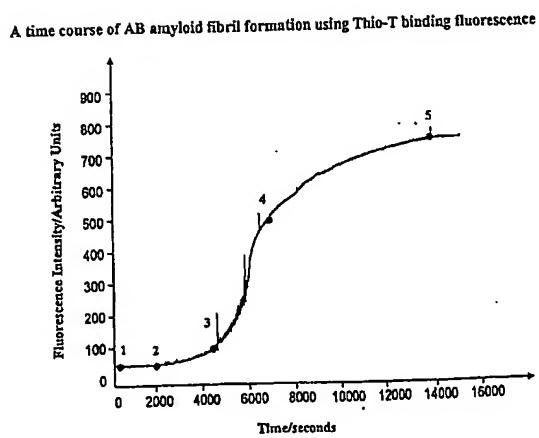


FIGURE 5A

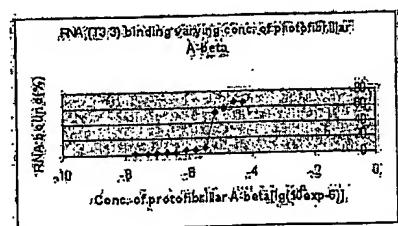


Fig. 2

5 FIGURE 5B

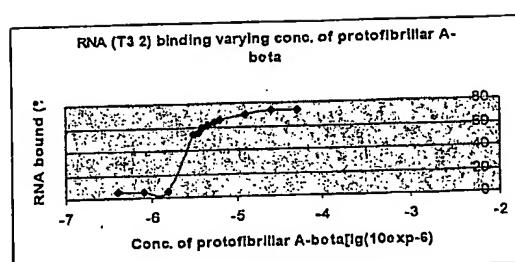
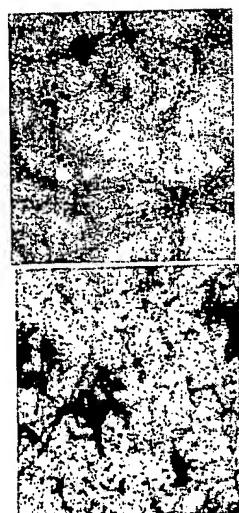
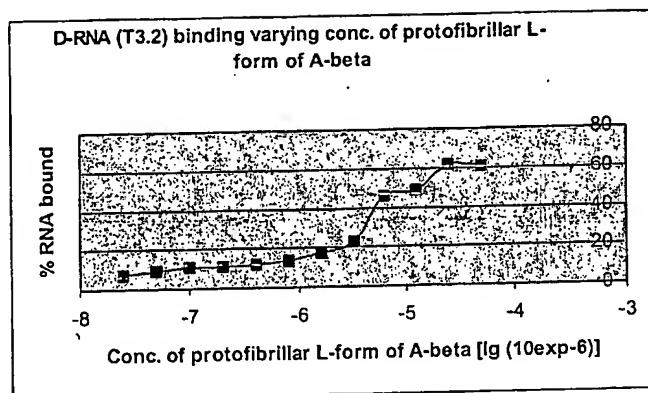


FIGURE 6



5

FIGURE 7

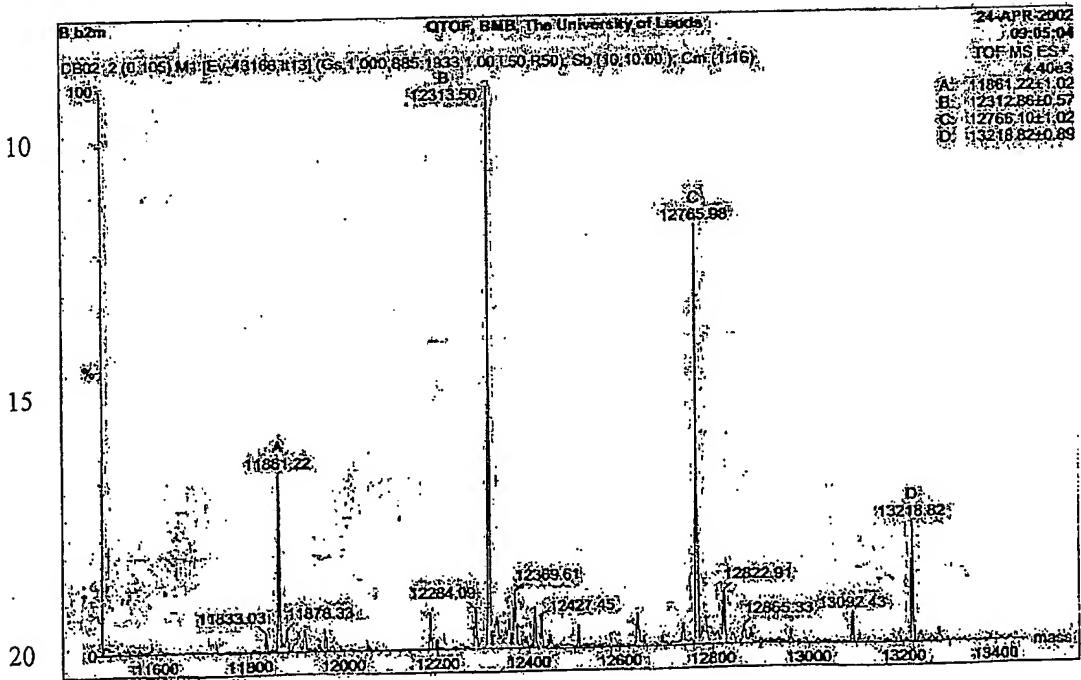


FIGURE 8

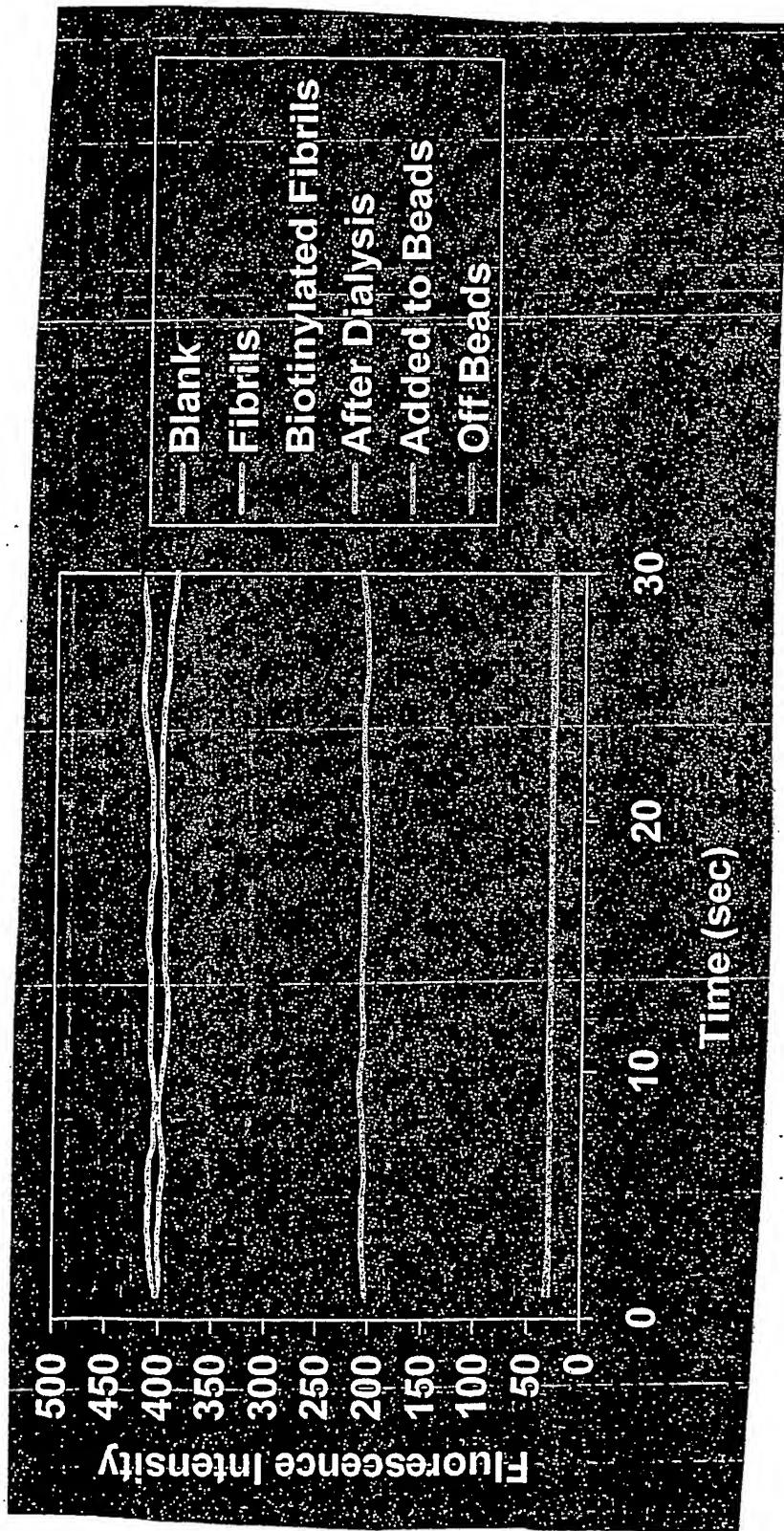


FIGURE 9

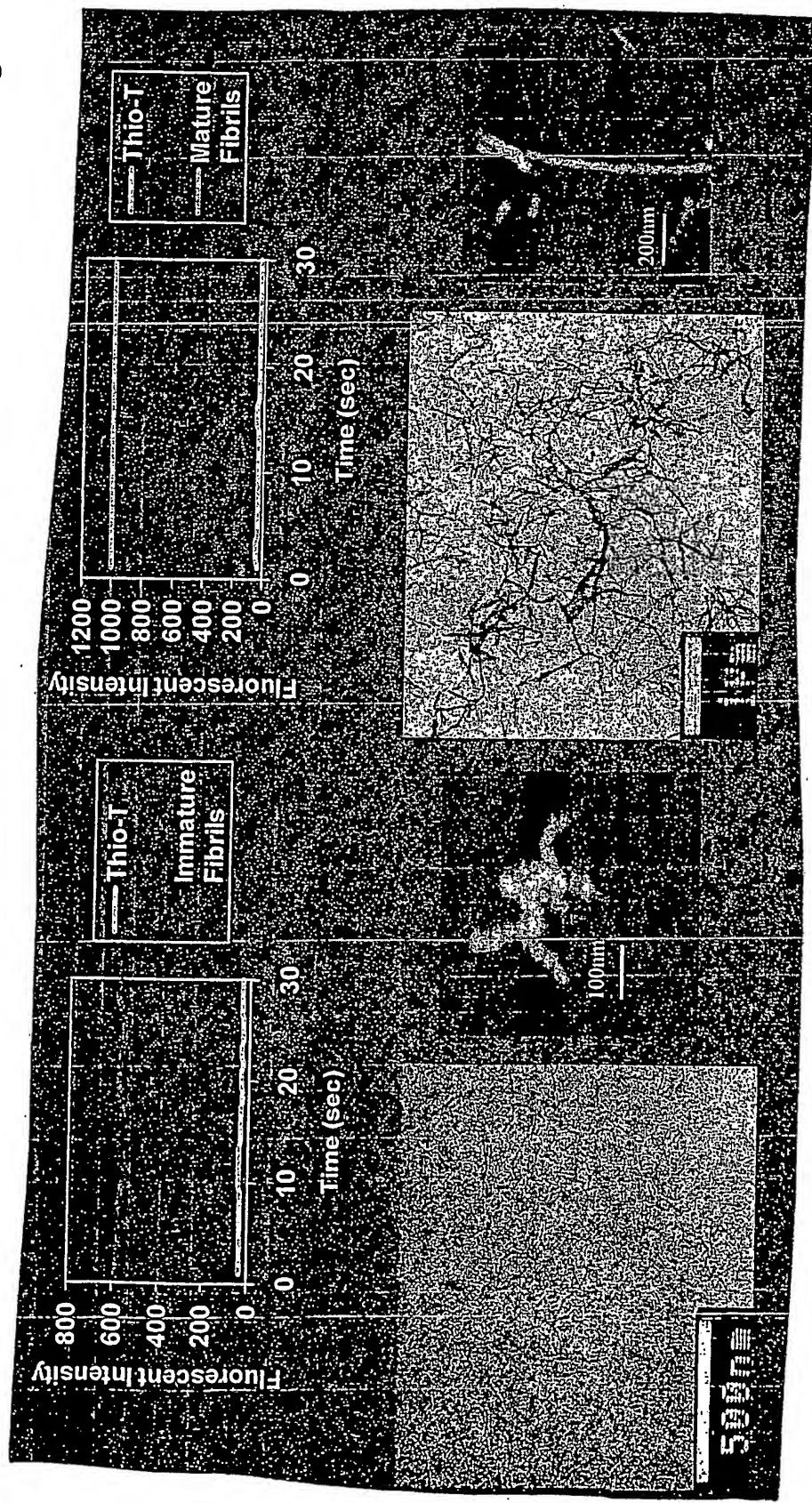


FIGURE 10

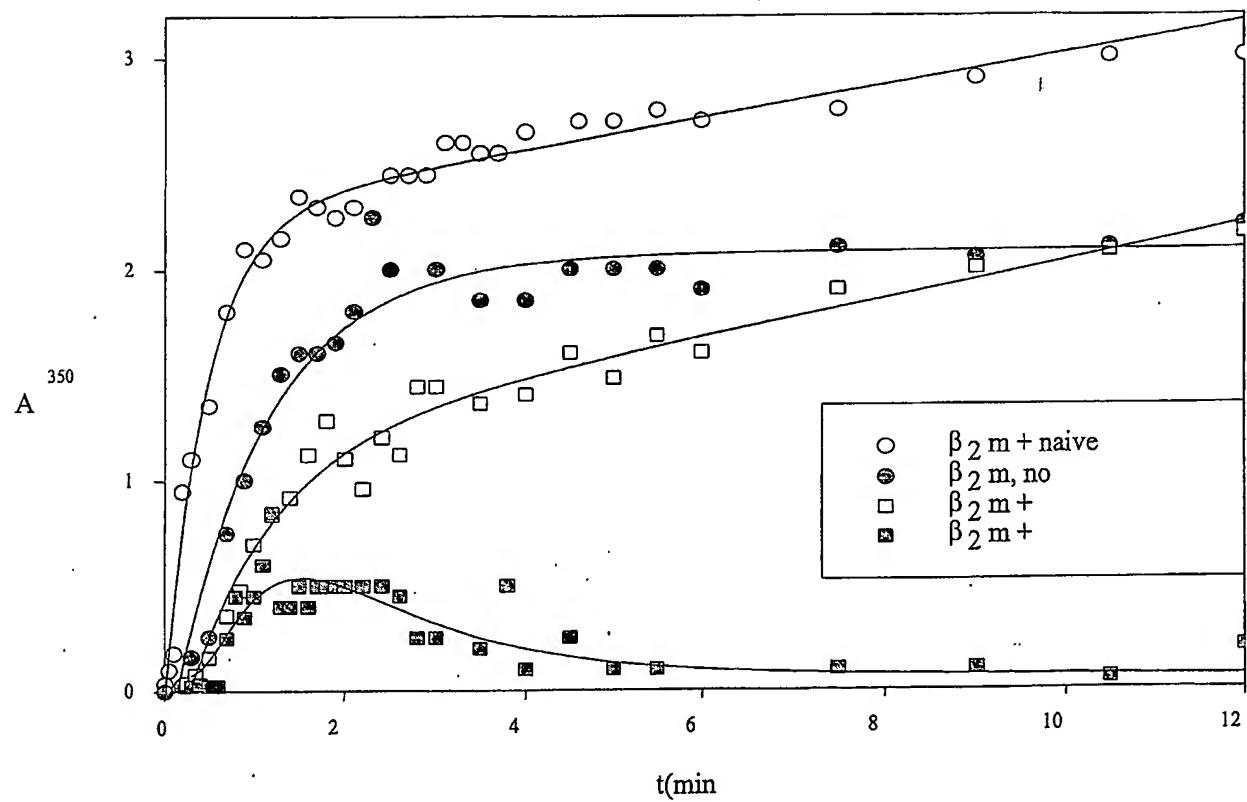
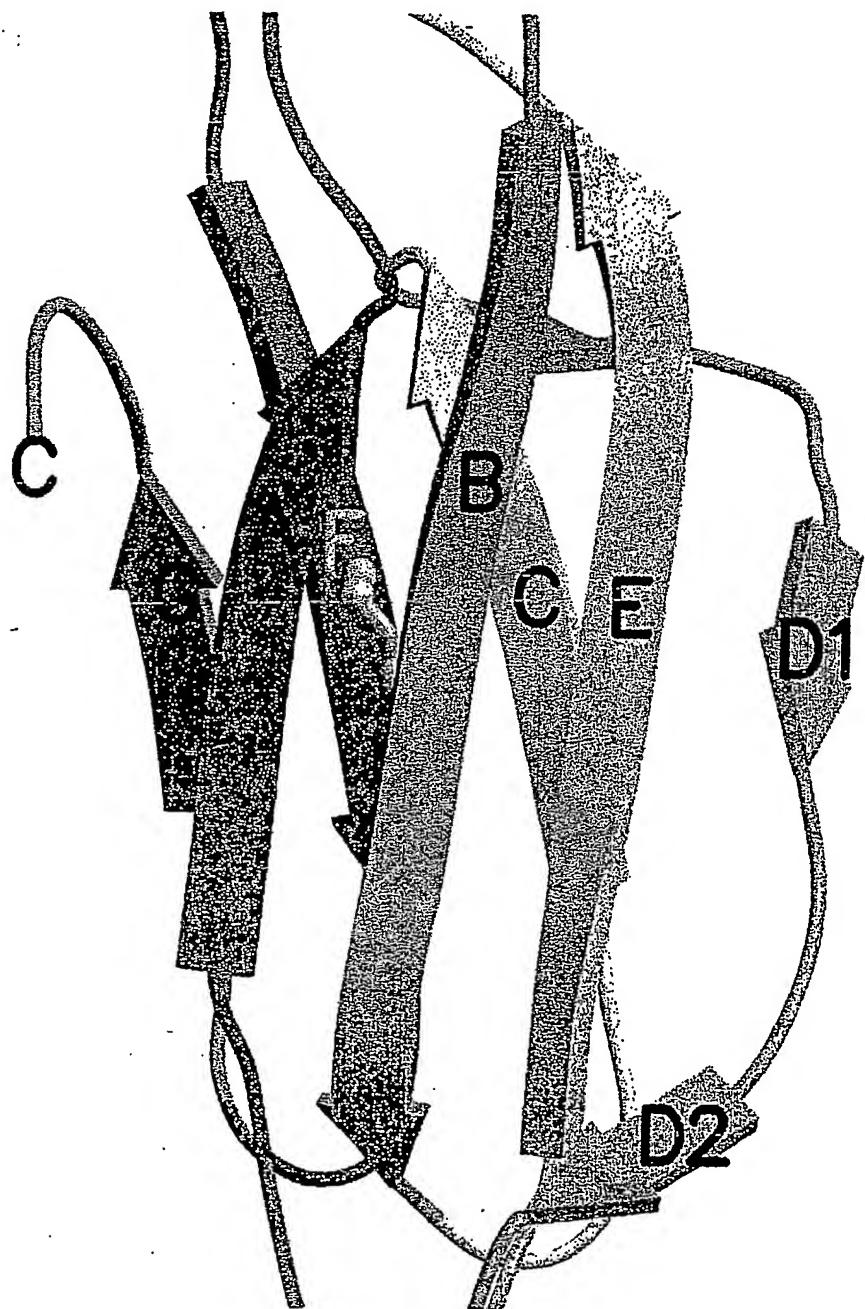
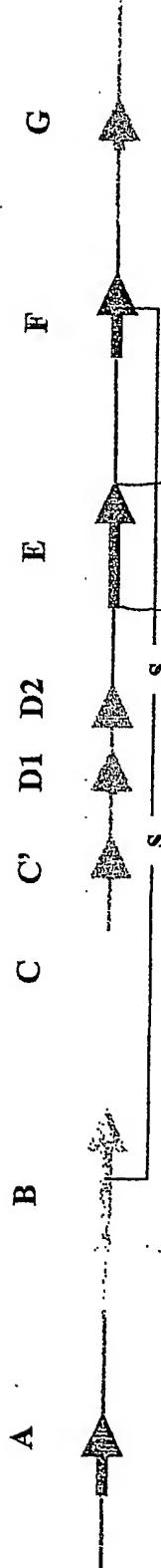


FIGURE 11





IQRTPKIQVYSRHPAENGKSNFILCYVSGEHPSDIEVTLKNGERIEKEVHSDLSFSKDWSEFLLYYTERTPTEKDEYACRVNHWTLSQPKIVKWDRDM (SEQ ID NO: 114)

1. IORTPKIQVYSRHAE¹⁶ (SEQ ID NO: 115)
17. IGIKSNELNQYXSYG¹⁷ (SEQ ID NO: 116)

DANGERRIEKVEHSDISSESKD⁵⁹ (SEQ ID NO: 117)
59DWSEFLYXYTEFT⁷¹ (SEQ ID NO: 112)
59DWSEFLYXYTEFTPTERDEY⁷⁹ (SEQ ID NO: 113)
72PTERDEYACRVTNHVT⁸⁶ (SEQ ID NO: 118)
(SEQ ID NO: 119) ...LSQPKIVKWDRD⁹⁴"

FIGURE 12

19

PCT Application

GB0304798

